EXCERPTUM

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From the Pharmacological Institute of the University of Uppsala.

Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes.

IV. The Effect of Carotid Ligation and Cervical Sympathectomy in Guinea-pigs on the Ascorbic Acid Content of the Aqueous Humour at Varying Plasma Levels.

By

ERIK LINNÉR.

Received 12 March 1952.

Diss Uppsala

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In the present paper the concentration of ascorbic acid in the aqueous humour of guinea-pigs has been used as an indicator of circulatory changes in the ciliary processes at plasma-concentrations varying over a wide range. The same principles were followed as in the investigations in rabbits (Linnér 1951 a, 1951 b, 1952 a, 1952 c). The blood flow through one eye was changed by means of unilateral carotid ligation, or by pre-ganglionic section of the cervical sympathetic and a study made of the ratio between the concentration of ascorbic acid in the aqueous humour of the two eyes.

Materials and Methods.

Male guinea-pigs weighing 400—900 g were used. They were kept on a diet of hay, oats, turnips, and water, *ad lib.* The operations on the carotid artery, or the cervical sympathetic, and the sampling of the aqueous humour, were carried out under ether anaesthesia. The guinea-pigs in one group were given a subcutaneous infusion, over a period of 6 hours, of a freshly-made 2.2 per cent solution of ascorbic acid with pH adjusted to 5.5—6.5 by means of NaOH. The rate of infusion was 1 ml per hour. In order to get a higher ascorbic acid-level some animals

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Male guinea-pigs weighing 400—900 g were used. They were kept on a diet of hay, oats, turnips, and water, *ad lib.* The operations on the carotid artery, or the cervical sympathetic, and the sampling of the aqueous humour, were carried out under ether anaesthesia. The guinea-pigs in one group were given a subcutaneous infusion, over a period of 6 hours, of a freshly-made 2.2 per cent solution of ascorbic acid with pH adjusted to 5.5—6.5 by means of NaOH. The rate of infusion was 1 ml per hour. In order to get a higher ascorbic acid-level some animals

were given ascorbic acid subcutaneously 18—20 hours before sampling the aqueous humour. The technique, which was also used in earlier investigations has been described in detail in previous papers (LINNÉR 1952 a, 1952 c). Standard statistical methods were used according to the formulas given in BONNIER-TEDIN (1940). The standard error of a single determination was calculated by means of the difference between two determinations on every sample (DAHLBERG 1940).

Results.

Table 1 shows the standard error of a single determination.

Table 1.

The standard error of a single determination calculated by means of the difference between two determinations on every aqueous- or plasma-sample.

Double determinations have been made on each of 40 samples of aqueous humour in guinea-pigs saturated with ascorbic acid and 20 samples of plasma from 20 different guinea-pigs in each group at different saturation levels.

Ascorbic acid in mg%		
Aqueous humour	Plasma	
	Low concentration	High concentration
m = 29.77 s = 0.63 n = 40	m = 0.51 s = 0.02 n = 20	m = 14.73 s = 0.28 n = 20

Symbols: m = arithmetic mean
s = standard error of a single determination
n = number of samples

Table 2 shows the ratio between the ascorbic acid content in the aqueous humour of the eye influenced by carotid ligation, and that of the control eye. In the whole series of 26 guinea-pigs the mean ratio is 0.88 ± 0.02. The guinea-pigs with low ascorbic acid levels (1—7 mg per cent in the control eye) have a corresponding ratio of 0.86, and the guinea-pigs with high ascorbic acid levels (11—15 mg per cent in the control eye) have a ratio of 0.91. The difference between them is not statistically significant ($0.3 < P < 0.4$).

Figure 1 shows a scatter diagram of the results, with the control eye as the abscissa. The equation of the regression-line was calculated to:

$$Y = -0.25 + 0.92\,x$$

Table 2.

The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels below the saturation level.

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

	Ascorbic acid in mg%			Ratio: Ligated / Non-ligated
	Aqueous humour		Plasma	
	Non-ligated side	Ligated side		
The whole series of 26 guinea-pigs	m = 8.51 s = 3.95 e = 0.78 n = 26	m = 7.60 s = 3.72 e = 0.73 n = 26	m = 0.52 s = 0.28 e = 0.05 n = 26	m = 0.88 s = 0.09 e = 0.02 n = 26
Group of guinea-pigs with a low ascorbic acid level (1—7 mg% in the control eye)	m = 3.83 s = 1.73 e = 0.58 n = 9	m = 3.38 s = 1.62 e = 0.54 n = 9	m = 0.38 s = 0.08 e = 0.03 n = 9	m = 0.86 s = 0.11 e = 0.04 n = 9
Group of guinea-pigs with a high ascorbic acid level (11—15 mg% in the control eye)	m = 12.61 s = 0.93 e = 0.31 n = 9	m = 11.50 s = 1.43 e = 0.48 n = 9	m = 0.61 s = 0.25 e = 0.08 n = 9	m = 0.91 s = 0.08 e = 0.03 n = 9

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 3 shows the ratio between the ascorbic acid content in the aqueous humour of the eye with a cut cervical sympathetic and that in the control eye. In the whole series of 36 guinea-pigs the mean ratio is 1.23 ± 0.03. The guinea-pigs with low ascorbic acid levels (2—8 mg per cent in the control eye) have a corresponding ratio of 1.27, and the guinea-pigs with high ascorbic acid levels have a ratio of 1.20. The difference between them is not statistically significant ($0.2 < P < 0.3$).

Figure 2 shows a scatter diagram of the results, with the control eye as the abscissa. The equation of the regression-line was calculated to:

$$Y = 0.61 + 1.16\ x$$

A more far-reaching statistical analysis has not been made for the same reasons as in the earlier paper (Linnér 1952 a). As in the experiments on rabbits, it seems reasonable to say that the two scatter diagrams in figures 1 and 2 are best represented by a straight regression-line.

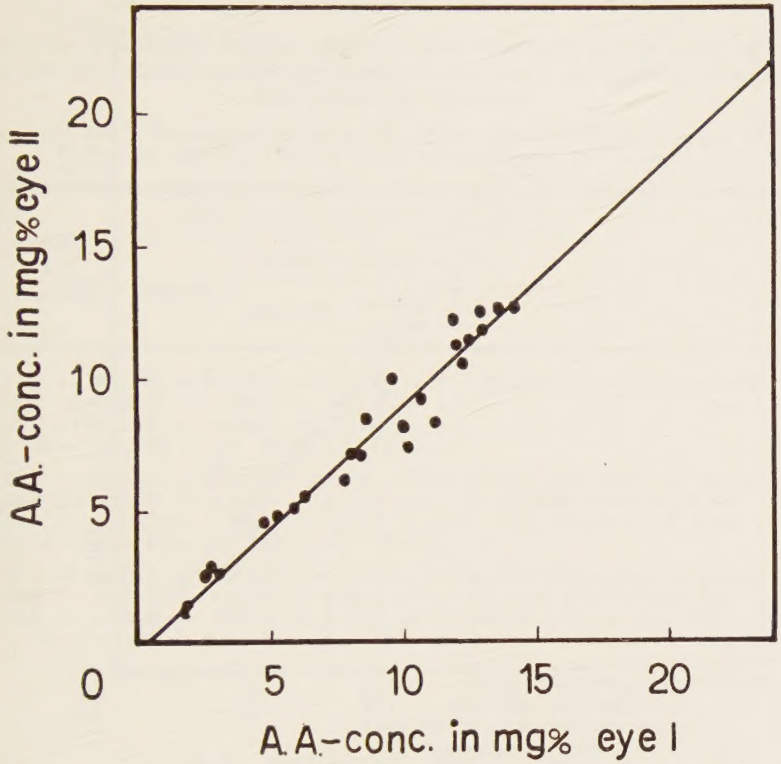

Fig. 1. The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour, at ascorbic acid levels below the saturation level. The carotid artery was ligated 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye influenced by carotid ligation. The concentration in mg ascorbic acid per 100 ml.

Data are also available for another acid, para-amino-hippuric acid (PAHA) which is transferred across the blood-aqueous barrier at a slow rate. This probably means that the rate of blood flow is not the limiting factor for the rate of transfer of PAHA through the blood-aqueous barrier. 37 guinea-pigs with the cervical sympathetic cut on one side were given a subcutaneous infusion of PAHA over a period of 6 hours. The ratio between the two eyes was 1.12 ± 0.07 and calculated as the median 1.04 (Bárány 1952). This value is probably greater than 1 even if the difference from 1 is not statistically quite significant. At a decrease in the rate of flow of aqueous humour the concentration of PAHA in the aqueous humour would increase if the transfer

Table 3.

The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels below the saturation level.

The cervical sympathetic was cut 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

	Ascorbic acid in mg%			Ratio: Cut / Non-cut
	Aqueous humour		Plasma	
	Non-cut side	Cut side		
The whole series of 36 guinea-pigs	m = 10.57 s = 4.92 e = 0.82 n = 36	m = 12.83 s = 5.75 e = 0.96 n = 36	m = 0.77 s = 0.74 e = 0.12 n = 36	m = 1.23 s = 0.15 e = 0.03 n = 36
Group of guinea-pigs with a low ascorbic acid level (2—8 mg% in the control eye)	m = 5.56 s = 1.54 e = 0.41 n = 14	m = 6.93 s = 1.74 e = 0.46 n = 14	m = 0.46 s = 0.16 e = 0.04 n = 14	m = 1.27 s = 0.23 e = 0.06 n = 14
Group of guinea-pigs with a high ascorbic acid level (13—21 mg% in the control eye)	m = 15.91 s = 2.58 e = 0.71 n = 13	m = 19.06 s = 2.83 e = 0.79 n = 13	m = 1.02 s = 1.07 e = 0.30 n = 13	m = 1.20 s = 0.06 e = 0.02 n = 13

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

of PAHA across the blood-aqueous barrier were not decreased to the same extent as the rate of flow of aqueous humour.

As shown by KINSEY (1947) and LANGHAM (1949), the saturation of the secretory part of the eye in rabbits is reached at a plasma ascorbic acid level of about 3 mg per cent. Figure 3 shows a scatter diagram with the plasma ascorbic acid concentration in guinea-pigs on the abscissa and the ascorbic acid concentration of the aqueous humour in the control eye on the ordinate. From this scatter diagram it appears that the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier in guinea-pigs is reached at a plasma ascorbic acid level of about 2 mg per cent. The concentration of ascorbic acid in the aqueous humour at saturation is about 30 mg per cent against 50 mg per cent in rabbits.

As in the experiments on rabbits, the saturation level of the guinea-pigs in two groups was exceeded for 6 hours. The plasma

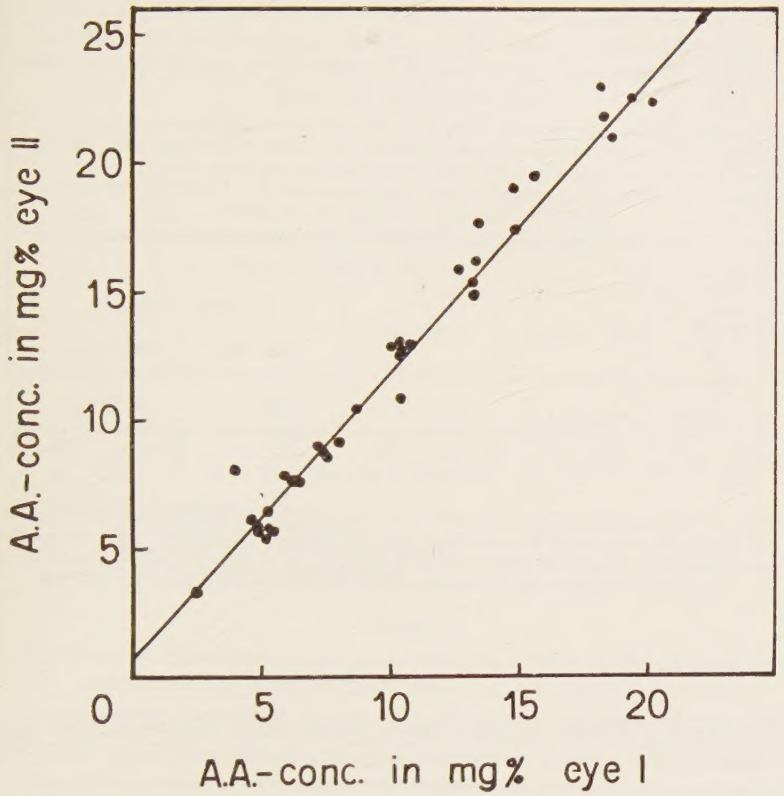

Fig. 2. The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour, at plasma ascorbic acid levels below saturation level. The sympathetic was cut 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye with cut sympathetic. The concentration in mg ascorbic acid per 100 ml.

ascorbic acid level was throughout higher than 2 mg per cent. As can be seen in table 4, there is no difference between the concentration of ascorbic acid in the eye influenced by carotid ligation and in the control eye. On the other hand, as shown in table 5, the ratio between the ascorbic acid content in the aqueous humour of the eye with a cut cervical sympathetic and that in the control eye is 1.08 ± 0.008.

As in the experiments on rabbits where one cervical sympathetic was cut some guinea-pigs were given Dibenamine in order to compare the activity of this substance alone with the effect of sympathectomy plus Dibenamine. The cervical sympathetic was cut on one side and Dibenamine was injected subcutaneously in

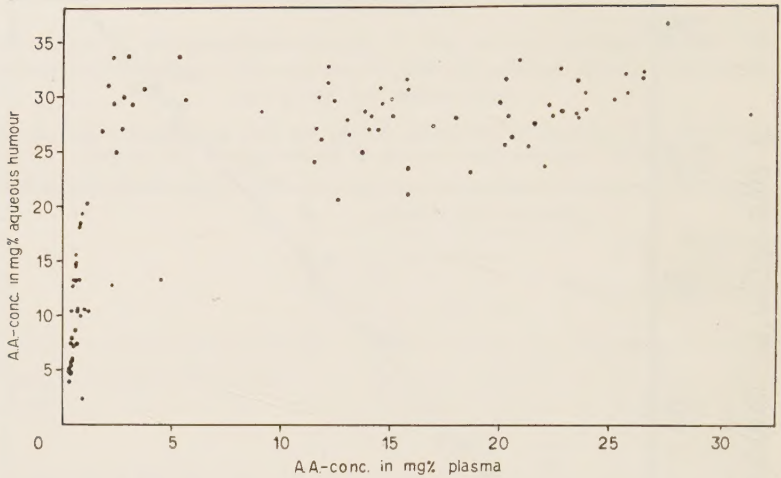

Fig. 3. The concentration of ascorbic acid in the aqueous humour at varying plasma levels showing that the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier in guinea-pigs is reached at a plasma ascorbic acid level of about 2 mg per cent. Concentrations in mg ascorbic acid per 100 ml.

Table 4.

The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels above the saturation level.

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. The saturation level was exceeded by means of subcutaneous infusion of an ascorbic acid solution over a period of 6 hours. Plasma I was removed after 3 hours and plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

Ascorbic acid in mg%				Ratio: Ligated / Non-ligated
Aqueous humour		Plasma I	Plasma II	
Non-ligated side	Ligated side			
m = 29.36 s = 2.93 e = 0.61 n = 23	m = 29.24 s = 2.97 e = 0.62 n = 23	m = 15.36 s = 2.79 e = 0.58 n = 23	m = 22.58 s = 2.94 e = 0.61 n = 23	m = 1.00 s = 0.04 e = 0.009 n = 23

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 5.

The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels above the saturation level.

The cervical sympathetic was cut 18—20 hours before removal of the aqueous humour. The saturation level was exceeded by means of subcutaneous infusion of an ascorbic acid solution over a period of 6 hours. Plasma I was removed after 3 hours and Plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

Ascorbic acid in mg%				Ratio: Cut / Non-cut
Aqueous humour		Plasma I	Plasma II	
Non-cut side	Cut side			
m = 27.59 s = 2.97 e = 0.56 n = 28	m = 29.87 s = 2.98 e = 0.56 n = 28	m = 8.96 s = 3.25 e = 0.68 n = 23	m = 14.54 s = 3.08 e = 0.58 n = 28	m = 1.08 s = 0.04 e = 0.008 n = 28

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 6.

The effect of Dibenamine and pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour.

The sympathetic was cut on one side and Dibenamine was given subcutaneously 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

	Ascorbic acid in mg%			
	Aqueous humour		Plasma	Ratio: Cut / Non-cut
	Non-cut side	Cut side		
Guinea-pigs given 10—20 mg/kg Dibenamine	m = 14.47 s = 5.25 e = 1.46 n 13	m = 15.67 s = 5.52 e = 1.53 n 13	m = 0.68 s = 0.44 e = 0.12 n 13	m = 1.09 s = 0.06 e = 0.02 n 13
Guinea-pigs given 50 mg/kg Dibenamine	m = 11.48 s = 5.95 e = 1.49 n = 16	m = 12.09 s = 6.00 e = 1.50 n = 16	m = 0.67 s = 0.42 e = 0.10 n = 16	m = 1.06 s = 0.06 e = 0.02 n = 16
Guinea-pigs given 100 mg/kg Dibenamine	m = 10.63 s = 4.68 e = 1.00 n = 22	m = 10.78 s = 4.73 e = 1.01 n = 22	m = 0.50 s = 0.12 e = 0.03 n = 21	m = 1.02 s = 0.07 e = 0.01 n = 22

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 7.

The effect of pre-ganglionic section of the cervical sympathetic on the amount of aqueous humour which could be obtained from the anterior chamber.

The amount of aqueous humour in mg.

Amount of aqueous humour in mg	
Non-cut side	Cut side
m = 43.12	m = 41.26
s = 21.25	s = 22.36
e = 2.24	e = 2.36
n = 90	n = 90

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

6—8 different places. 18—20 hours later the aqueous humour was sampled. Table 6 shows that the ascorbic acid ratio is 1.09 when 10—20 mg/kg Dibenamine has been given. When 50 mg/kg has been given the ratio drops to 1.06, and with 100 mg/kg the ratio is 1.02. These findings, which differ from those in rabbits, will be discussed in the theoretical part.

Table 7 shows that there is no statistically significant difference in the amount of aqueous humour which was obtained from the eye with the cut cervical sympathetic and that from the control eye.

Theoretical Part.

T = rate of transfer of ascorbic acid from plasma to aqueous humour.
T_{max} = maximal rate of transfer of ascorbic acid from plasma to aqueous humour.
Fl_{pl} = rate of plasma flow through the ciliary processes.
Fl_{aq} = rate of flow of aqueous humour.
C_{art} = the concentration of ascorbic acid in the arterial plasma.
C_{ven} = the concentration of ascorbic acid in the venous plasma leaving the ciliary processes.
C_{aq} = the concentration of ascorbic acid in the aqueous humour.

Subscript 1 stands for the control eye.

Subscript 2 stands for the eye influenced by carotid ligation or cervical sympathectomy.
Subscript 'sat' = plasma ascorbic acid level exceeds the saturation level.

$$q = \text{the ratio } \frac{C_{aq_2}}{C_{aq_1}}$$

In all the experiments on rabbits and guinea-pigs analysed in detail here, the ascorbic acid concentration in the aqueous humour was determined. As the lens can be supposed to be of small or no importance for the ascorbic acid concentration of the aqueous humour in the species investigated here, the following equation can be used:

$$C_{aq} = \frac{T}{Fl_{aq}} \tag{1}$$

In both rabbits and guinea-pigs $\frac{C_{aq_2}}{C_{aq_1}}$ was determined after unilateral carotid ligation or sympathectomy. In each of the four experimental series the ratio

$$\frac{C_{aq_2}}{C_{aq_1}} = q \tag{2}$$

is essentially constant and statistically different from 1, when C_{art} varies over a wide range below T_{max}. This result can be produced by changes either in T or in Fl_{aq} (equation 1). Supposing that the ascorbic acid is neither stored nor used up in the epithelium, the following equations are obtained:

$$T = Fl_{pl} (C_{art} - C_{ven}) \tag{3}$$

where a slight decrease in the rate of plasma flow through the ciliary processes is neglected.

$$q = \frac{Fl_{pl_2}}{Fl_{pl_1}} \times \frac{C_{art_2} - C_{ven_2}}{C_{art_1} - C_{ven_1}} \times \frac{Fl_{aq_1}}{Fl_{aq_2}} \tag{4}$$

Since the blood flowing to both eyes can be expected to have the same ascorbic acid concentration $C_{art_1} = C_{art_2}$, thus the subscripts 1 and 2 to C_{art} can be omitted.

In each series $\frac{Fl_{pl_2}}{Fl_{pl_1}}$ can be regarded as essentially constant, since there is no reason why the change in Fl_{pl} produced by the operations, should vary at varying ascorbic acid levels. According to BÁRÁNY (1951), Fl_{aq} in guinea-pigs, unless highly scorbutic, is not influenced by variations in the ascorbic acid level. Thus in the present investigation $\frac{Fl_{aq_1}}{Fl_{aq_2}}$ is regarded as constant in each series despite varying ascorbic acid levels. From this it follows that

$$Q = \frac{C_{art} - C_{ven_2}}{C_{art} - C_{ven_1}} = \text{constant} \tag{5}$$

in each series, regardless of variations in C_{art}.

A mathematical analysis of the rate of transfer of ascorbic acid from plasma to aqueous humour will now be made and compared to the experimental results. At first we start with a schematic and simplified model of the ciliary processes.

According to BAURMANN (1930) the capillaries of the ciliary processes of the rabbit are arranged in single layers between epithelial sheets. As a first approximation each capillary may therefore be considered to be supplied with its own epithelial layer. The blood flowing along this vessel is gradually extracted and the concentration of ascorbic acid drops.

We assume schematically that the epithelial cells transfer ascorbic acid from plasma to aqueous humour in the following way. The velocity of transfer by the epithelial cells per unit of length and time is called V(C), to indicate clearly that it is dependent on the local plasma concentration C. A certain minimal concentration of ascorbic acid in the plasma is needed for the epithelial cells to be able to start the transfer of ascorbic acid from plasma to aqueous humour; this threshold concentration is here called C_t. For increasing values of C above C_t the function V(C) increases up to a saturation value, V_s; this value is reached at a concentration of ascorbic acid in the plasma here called C_s. For C larger than C_s is $V(C) = V_s$. To simplify the following calculations it is assumed that V(C) increases linearly to the maximal value V_s.

The capillaries of the ciliary processes are at first assumed to be replaced by one vessel of the length l. A coordinate x is introduced along the vessel: $x = 0$ at the beginning of the vessel and $x = l$ at its end. The concentration C(x) will consequently decrease along the vessel, which means that its derivative is negative. By means of the rate of transfer introduced above, V(C), the following equation is obtained for the derivative of the concentration along the vessel:

$$\frac{dC(x)}{dx} = -\frac{V[C(x)]}{f}, \tag{6}$$

where f is the rate of plasma flow in unit time.

This equation shows how the concentration of ascorbic acid decreases along the vessel from the initial value C_{art} to the final value C_{ven}. We shall now consider how the concentration of ascorbic acid decreases along the vessel with different values of C_{art}.

Alt. A: $C_{art} \leq C_t$.

In this case C(x) is constantly $= C_{art}$.

Alt. B: $C_t < C_{art} \leq C_s$

i. e. no part of the transfer mechanism is saturated. From equation (6) one obtains:

$$C(x) = C_t + (C_{art} - C_t)\, e^{-\frac{V_s x}{f(C_s - C_t)}} \tag{7}$$

which yields

$$C_{ven} = C_t + (C_{art} - C_t)\, e^{-\frac{V_s l}{f(C_s - C_t)}}. \tag{8}$$

The concentration of ascorbic acid in the plasma here decreases exponentially along the vessel.

$$\text{Alt. C: } C_s < C_{art} \leq C_s + \frac{V_s\, l}{f}$$

i. e. part of the transfer mechanism is saturated *e. g.* up to $x = x_o$. This is the point where $C(x)$ has decreased to C_s. For the interval $(0, x_o)$ the equation now becomes:

$$C(x) = C_{art} - \frac{V_s\, x}{f} \qquad (9)$$

i. e. the concentration of ascorbic acid in the plasma decreases linearly along the vessel. In the interval (x_o, l) the concentration of ascorbic acid decreases exponentially as in alt. B. The explicit expression for this interval is unnecessary to the present discussion.

$$\text{Alt. D: } C_{art} > C_s + \frac{V_s\, l}{f}$$

i. e. the transfer mechanism is completely saturated and the concentration decreases linearly in the whole interval.

Then

$$C_{ven} = C_{art} - \frac{V_s\, l}{f}\,. \qquad (10)$$

We now return to the ratio Q in equation (5) to substitute there the expressions derived from the mathematical analysis and we shall study Q as a function of C_{art}. The two different rates of plasma flow are called f_1 and f_2. It is not regarded as necessary to give the results explicitly but for the alternatives B and D.

Alt. B: (the transfer mechanism completely unsaturated)

$$Q = \frac{C_{art} - C_{ven_2}}{C_{art} - C_{ven_1}} = \frac{1 - e^{-\frac{V_s\, l}{f_2\,(C_s - C_t)}}}{1 - e^{-\frac{V_s\, l}{f_1\,(C_s - C_t)}}} = \text{constant} \qquad (11)$$

Alt. D (the transfer mechanism completely saturated):

$$Q = \frac{f_1}{f_2} = \text{constant} \qquad (12)$$

In alt. B (equation 11) the value of Q is nearer unity than in alt. D (equation 12). In the interval of alt. C Q changes monotonously from the constant value of alternative B to the constant value of alternative D.

The same type of the function Q is obtained if the one-vessel-model is replaced by a system of vessels arranged in parallel varying in length and diameter.

From the theory presented above it follows that, at varying C_{art} below saturation, Q is exactly constant only in alt. B, *i. e.* when the transfer mechanism is completely unsaturated. From a physiological point of view, however, alt. B seems improbable even if it cannot be excluded by means of data now available. Alt. B requires that the epithelium at the beginning of the vessels remains unsaturated in spite of the fact that C_{art} is as high as 2/3 of complete saturation. On the other hand it is much more probable that alt. C is true, *i. e.* part of the transfer mechanism is saturated. According to the theory, however, Q is not constant in alt. C.

It should be remembered that q and consequently also Q is found to be approximately constant in all the four experimental series at varying C_{art} below saturation. Of course this does not exclude certain deviations, so small that they cannot be observed on account of statistical variations.

If the extraction is so complete that both C_{ven_1} and C_{ven_2} decrease to a value near the threshold value C_t alt. C in the theory agrees with the experimental values. This means that Q approximates to unity. There is no direct proof for this alternative but it must be regarded as most probable from a physiological point of view.

If we consider Q to approximate unity it will mean that equation (4) assumes the following simple form, valid for the plasma concentration below saturation:

$$q = \frac{Fl_{pl_2}}{Fl_{pl_1}} \times \frac{Fl_{aq_1}}{Fl_{aq_2}} . \qquad (13)$$

At plasma concentration above saturation the equation will be replaced by the following:

$$q_{sat} = \frac{Fl_{aq_1}}{Fl_{aq_2}} \times \frac{T_{max_2}}{T_{max_1}} . \qquad (14)$$

Here the maximal rate of transfer from plasma to aqueous humour is introduced into the equation. This equation is generally valid and independent of the hypothesis concerning Q.

To begin with we shall consider the case where $T_{max_2} = T_{max_1}$. After carotid ligation in rabbits and guinea-pigs and after cervical sympathectomy in rabbits q_{sat} has been shown not to vary significantly from unity. From equation (14) follows directly that $\frac{Fl_{aq_1}}{Fl_{aq_2}} = 1$. In these three cases equation (13) will consequently assume the following form:

$$q = \frac{Fl_{pl_2}}{Fl_{pl_1}} . \qquad (15)$$

Thus, ascorbic acid can be used for measuring changes in the rate of plasma flow through the ciliary processes.

The hypotheses made in the course of these calculations will now be analysed in their connection with the experimental results.

Sympathectomy, whereby the depressor nerve was damaged by the technique used in these experiments, and carotid ligation, caused a slight increase in the blood pressure and some peripheral vasoconstriction. Because of this the control eye cannot be regarded as completely uninfluenced by the operation. However, since this influence is the same in both eyes, it is of no importance for q. For this reason it is sufficient to discuss only the changes in the eye on the ligated or sympathectomized side.

We start with equation (14) and the experimental results obtained above saturation. After carotid ligation in rabbits the ratio q_{sat} was found to be 1.01 ± 0.008 and in guinea-pigs 1.00 ± 0.009. After sympathectomy q_{sat} was found to be 1.01 ± 0.01 in rabbits, whereas in guinea-pigs it deviated from unity (1.08 ± 0.008). Thus, in the three first mentioned series a ratio of one was found. If the two ratios of equation (14) are not influenced by the operations, *i. e.* if $T_{max_2} = T_{max_1}$ and $Fl_{aq_2} = Fl_{aq_1}$, then it follows that $q_{sat} = 1$.

There is, however, a possibility that the two ratios of equation (14) are influenced in such a way that q_{sat} still remains unity. This might be the case *e. g.* if the carotid ligation causes a decrease of the mean-pressure-head which changes the pressure-flow diagram, whereby vessels of a certain caliber collapse completely excluding a certain part of the ciliary processes but leaving the remainder essentially uninfluenced. A similar change in the ratios might occur after a sympathectomy if new areas of blood vessels, which before were completely excluded from the circulation, become opened while the other areas remain uninfluenced. However, a great many facts are known from experiments on the physiology of the circulation, which indicate that the effects caused by such circulatory changes as carotid ligation or sympathectomy are not limited to the above mentioned exclusion or inclusion of a certain part of the vascular bed.

Thus, the results obtained with ascorbic acid alone are not sufficient to decide whether Fl_{aq} and T_{max} remain unchanged or not. A simultaneous measurement in each eye separately of C_{aq} and Fl_{aq} would be necessary to give the real change. At present, however, there are no suitable methods available for studies of this kind in animals. In the single human eye, Fl_{aq} can be esti-

mated by means of Goldman's fluorescein method (Goldmann 1950).

Thus, by means of the results obtained with ascorbic acid alone it is not possible to say if Fl_{aq_2} is changed. There is, however, a good agreement between the value of the ratio q below saturation level in rabbits, 0.83 (Linnér 1952 a), and the ratio between the amount of blood escaping from an opened vortex vein on the ligated and on the non-ligated side, 0.81 (Linnér 1952 b). This speaks in favour of Fl_{aq_2} not being changed to any great extent by carotid ligation. Furthermore the rate of disappearance of sodium is only slightly decreased by carotid ligation (no statistically significant decrease: Bárány 1947; 7 ± 1.5 % Davson and Matchett 1951). It is probable therefore that the effect of carotid ligation in the rabbit il of the order of 25 % reduction in plasma flow through the ciliary processes.

There are no direct measurements of a similar kind concerning the effect of sympathectomy. It should, however, be observed that the ratio was found to be 1.02 ± 0.01 in a group of rabbits with unilateral cervical sympathectomy which were given Dibenamine. According to the discussion in a previous paper (Becker and Linnér 1952) this indicates that the effect of the cervical sympathetic on the ciliary processes in the rabbit is probably limited to vasoconstriction.

In contradistinction to the other experimental series it was found that $q_{sat} = 1.08 \pm 0.008$ in guinea-pigs after sympathectomy; below saturation the ratio q was found to be 1.23 ± 0.03. The reduction of this ratio from 1.23 to 1.08 is probably analogous to the reduction from 1.11 to 1.01 observed in the rabbits. But the ratio 1.08 above saturation which is statistically different from unity remains to be explained.

10—20 mg/kg body-weight of Dibenamine gave a ratio q of 1.09 ± 0.02; 50 mg/kg reduced q to 1.06 ± 0.02 and after doses as large as 100 mg/kg q was 1.02 ± 0.01.

The ratio obtained by means of p-amino-hippuric acid in 37 guinea-pigs was found to be 1.12 ± 0.07; the median was calculated to 1.04 (Bárány 1952). This value is probably greater than unity even if the difference is not statistically significant. This result might therefore speak in favour of some decrease in Fl_{aq_2}.

The ratios obtained after sympathectomy both at levels above saturation and with Dibenamine can be explained by a decrease

in Fl_{aq_2} or an increase in T_{max_2} and T_2. There are conceivably several complicated mechanisms which could cause such a result. Here an explanation will be offered, which seems to the author to be the most probable one. There is, however, no conclusive evidence to prove its correctness.

The innervation to the ciliary processes in guinea-pigs (but not in rabbits) might include sympathetic secretory fibres. These fibres would have to be of greater importance for the transfer of water than of ascorbic acid across the blood-aqueous barrier. Furthermore, larger doses of Dibenamine would be required to block the secretory impulses than the vasoconstrictor impulses to the ciliary processes.

This explanation is based on three separate hypotheses. The existence of secretory fibres to the ciliary processes has never been proved but is compatible with a large body of clinical observations showing a rise of intraocular pressure in connection with overactivity of the sympathetic nervous system (for references see Duke-Elder 1940).

There are some clinical observations showing that Dibenamine has a lowering effect on the intraocular pressure in some types of glaucoma (Christensen and Swan 1949, Bloomfield and Haimovici 1950, Newell, Ridgeway and Zeller 1951, Clark and Duggan 1951, Muller and Leopold 1951). Assuming that there are sympathetic secretory fibres to the ciliary processes in the human eye in the same way as has been assumed for guinea-pigs and that the secretory impulses could be blocked by Dibenamine such a blocking effect might play a rôle in lowering the intraocular pressure in glaucoma.

The second hypothesis is that the secretory fibres mainly activate those secretory elements which are responsible for the bulk of the aqueous. There is no positive evidence for or against this hypothesis but the present experiments as well as those of Bárány (1951) at least show that transfer of water is not intimately connected with transfer of ascorbic acid. Whether the two are effected by separate anatomical structures or not is an open question. The third hypothesis, finally, is that the secretory fibres are more resistant to Dibenamine than vasomotor fibres. There are many examples of such differences between various functional systems in the sensitivity to autonomic blocking agents. It should be mentioned however, that Uvnäs (1948) found the ordinary dose of 10—20 mg/kg Dibenamine sufficient for complete blocking of

the sympathetic secretory impulses to the submaxillary gland of the cat. One has, of course, to be cautious in drawing conclusions from the submaxillary gland in the cat to the ciliary processes in the guinea-pig. Furthermore, in the present experiments Dibenamine was injected subcutaneously, which might be less efficient than the intravenous, intramuscular or intraperitoneal injection used by UVNÄS.

It cannot be concluded with certainty from the experiments discussed above what changes in Fl_{aq} have resulted from carotid ligation or sympathectomy. As far as the sympathectomy in guinea-pigs is concerned there is good reason to believe that Fl_{aq_2} has decreased. When it comes to the experiments on rabbits and the carotid ligation in guinea-pigs the data now available speak in favour of a slight decrease of Fl_{aq}. According to equation (13) this means that q gives only a minimal value of the real change in Fl_{pl}. A final elucidation of these questions will depend upon simultaneous measurements of C_{aq} and Fl_{aq}.

Summary.

The concentration of ascorbic acid in the aqueous humour was investigated at varying plasma levels of ascorbic acid in male guinea-pigs, in one group after unilateral carotid ligation, and in another group after unilateral section of the cervical sympathetic. Over a wide range below saturation level the ratio between the concentrations in the aqueous humour of the two eyes was 0.88 ± 0.02 in the group where the carotid artery was ligated, and 1.23 ± 0.03 in the group where the cervical sympathetic was cut. After carotid ligation no difference was found between the two eyes above saturation level. After section of the cervical sympathetic, on the other hand, the ratio between the two eyes was found to be 1.08 ± 0.008 above saturation level. After unilateral section of the cervical sympathetic together with subcutaneous administration of 10—20 mg/kg body-weight Dibenamine the ratio between the two eyes was found to be 1.09 ± 0.02, after 50 mg/kg the ratio was 1.06 ± 0.02 and after 100 mg/kg 1.02 ± 0.01. After section of the cervical sympathetic no difference was found between the amounts of aqueous humour in the two eyes.

An analysis of the present results and those previously obtained in rabbits indicates that at concentrations below saturation ascorbic acid appears to be removed down to a threshold level from

the plasma flowing through the ciliary processes. This fact makes possible the use of ascorbid acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes.

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